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14. ABSTRACT

The primary mission of the Military Molecular Medicine Initiative (MMMI), a congressionally-supported military-civilian collaboration between WRAMC, Windber Medical Center(WMC)/Windber Research Institute (WRI) is to: 1) Teach, implement and study lifestyle changes added to "best" medical practices that promote cardiovascular health; 2) Identify patients at risk earlier by characterizing cardiovascular disease at the molecular disease stage and identify biomarkers predictive of sub-clinical CVD; and 3) Relate genomic/proteomic changes to the evolution of CVD risk factors in response to lifestyle changes in an effort to prevent, arrest or reverse CVD.

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Introduction

The epidemics of cardiovascular disease (CVD), Type II diabetes, and obesity generate a major share of the preventable costs of American health care. Currently, the American health care market place does not support preventive care that would save lives and costs associated with these problems. Healthcare costs are predicted to rise from 16% of the US GDP in 2005 to 30% of the GDP by 2025 if we fail to invest in prevention. The primary mission of the ***Military Molecular Medicine Initiative (MMMI),*** a congressionally-supported military-civilian collaboration between Walter Reed Army Medical Center (WRAMC) and Windber Medical Center (WMC)/Windber Research Institute (WRI) is to: 1) Teach, implement and study lifestyle changes added to "best" medical practices that promote cardiovascular health; 2) Identify patients at risk earlier by characterizing CVD at the "molecular" disease stage and identifying biomarkers predictive of subclinical CVD; and 3) Relate genomic/proteomic changes to the evolution of CVD risk factors in response to lifestyle changes in an effort to prevent, arrest or reverse CVD. Within these objectives, the MMMI will include: a) a comprehensive and innovative CVD risk factor assessment and prevention program in the military beneficiary population; b) advanced imaging methods for quantifying numerous aspects of heart health in military and other populations; c) an optimal healing environment for CVD patients; and d) an integrated statistical analysis of clinical and molecular data to identify patterns of CVD risk factors that will allow a unique and intensive collection of data at the clinical and molecular levels for heart disease, but with applicability and relevance in patients with other chronic diseases such as cancer, diabetes, metabolic syndrome and obesity. The heart disease data base will provide the ability to find novel disease markers, new treatment approaches, and provide a unique venue for future research.

Body

Task #1: Complete data analysis of "Non-Invasive Coronary Artery Disease Reversal" (CADRe) Study Protocol conducted at WRAMC.

Status: Study closed as of 12 September 2006. Major data analyses complete. Study results presented in Annual Report dated 24 August 2006.

The following manuscript is in progress:
Marshall, DA, Walizer, EM, Vernalis, MN & Taylor, AJ. Effect of a One-Year Lifestyle Intervention Program on Carotid Intima Media Thickness. (In preparation).

Task #2: Initiate "A Blood Repository for Analysis of Molecular Changes Associated with Cardiovascular Disease Development" protocol.

Status: Protocol withdrawn from WRAMC IRB on 23 Feb 07 at the request of Dr. Vernalis. The protocol was delayed because of difficulties approving the CRADA / MOU and since there has been a change in ICHP mission focus to more operationally related research projects.

Task #3: Initiate CADRe Five-Year Follow-up Protocol.

Study Design and Objectives:
This follow-up study will determine the persistence of healthy lifestyle behavioral changes and CVD risk factor control results after their original CADRe study participation. This study will continue as a longitudinal observational study where patients will have yearly follow-up visits at 1, 2, 3, 4, and 5 years after completion or expected completion of the CADRe Study. This study

will involve prospective collection of data, however, there will be no tests ordered that are not considered WRAMC Cardiology standard of care for the study population identified. Therefore, there are no risks involved with this study outside those of the standard of care treatment. Specific aims are to determine:

1. Persistence of lifestyle change behaviors in diet, exercise, and stress management
2. Coronary risk-factor control
3. Quality of Life

Hypothesis

Subjects who have been exposed to an intensive lifestyle change program will demonstrate long-term carryover of heart healthy characteristics including persistence of favorable lifestyle change behaviors and risk factor control.

Up to 163 male and female CADRe study participants, age 18 years or older, with subsequent completion of Phase 1 of the CADRe Study (3-month data collection) will be recontacted and invited to participate in this five (5) year follow-up study (post-study completion or expected completion). Because of the timing of this protocol submission, the earlier cohorts will not have as many yearly follow-up periods as the later cohorts (See Table 1). The final cohort of participants completed the CADRe study in April 2005.

Table 1. Projected Longitudinal Follow-Up of CADRe Study

Cohort #	Completers (≥ 12 weeks of intervention)	Dropouts (< 12 week exposure)	No longer available	Available to enroll	Available Follow-Up				
					1-Yr	2-Yr	3-Yr	4-Yr	5-Yr
1	7	3		7					X
2	16	2	1	15					X
3	15	5	1	14					X
4	18	2	1	17				X	X
5	12	1		12				X	X
6	19			19				X	X
7	15	5		15			X	X	X
8	12	4		12			X	X	X
9	9	2		9			X	X	X
10	10			10		X	X	X	X
11	11	1		11		X	X	X	X
12	10	4		10		X	X	X	X
13	12	5		12	X	X	X	X	X
Totals	166	34	3	163	12	43	79	127	163

Primary Outcome Measure - Heart Health Index (HHI)

A composite index of 7 heart healthy characteristics (BMI 18.5 – 25; LDL-cholesterol < 100 mg/dL; dietary fiber intake ≥ 25 gms/day; consumption of 5 or more fruits and vegetables per day; BP < 140/90 mmHg; regular exercise ≥ 150 min/week, and daily practice of CADRe program stress management techniques) was selected as the primary outcome measure since the main goal of this study is to assess the persistence of lifestyle change behaviors and risk factor control. The HHI, presented as a single score (range 0-7), will be assigned to each subject yearly. Additionally, each of the 7 heart healthy characteristics will be assessed independently as a continuous variable.

Secondary Outcomes
Several additional outcomes will be assessed including:

- Changes in modifiable CVD risk factors: blood pressure, body composition and fitness, lipid levels and glucose
- Other biochemical markers: C-reactive protein
- Quality of Life: SF-36

Status: WRAMC HUC approved this study on 23 May 2006. Protocol was approved by the US Army Medical Department Center and School, Clinical Investigations Regulatory Office (CIRO) on 2 November 06. The MRMC Memorandum of Deferral was received 22 Jan 07.

Two addendums were submitted for this protocol. Addendum #1 was submitted to WRAMC HUC on 15 September 2006 requesting the following changes: Addition of COL(ret) Marina Vernalis as Co-PI, deletion of Debra Marshall, MD as AI and modification of role and responsibilities of investigators. Addendum approval received 5 Jan 07.

Due to the delay in approval of this protocol and subsequent loss of subjects from Cohorts 1-3, Addendum #2 requested these subjects be contacted for a one-time data collection visit. Without these subjects, the sample size needed for this study would be impacted. This addendum was submitted on 30 Nov 06 and approved on 20 Dec 06.

A Change of PI from COL(Ret) Allen Taylor to COL Randolph Modlin was approved by WRAMC DCI on 3 December 2008. All appropriate documents have been changed to reflect the new PI.

Recruitment began on 1 Feb 07. An initial recruitment letter was mailed to 163 former CADRe Study 3-month completers known to be available for potential participation. A second mailing occurred on 1 Mar 07 to those potential participants who had not responded to the initial mailing. To date, 102 participants have responded (63%): 80 meet eligibility criteria and agreed to make a study visit; 2 are ineligible; 17 declined screening interview / participation; 2 are undecided about participation, and; 1 deceased. Of the 80 eligible patients who have agreed to make a study visit, 76 participants have provided informed consent and provided data for their initial study visit. Forty-five participants have provided at least one follow-up study visit.

Study participants are 66 yrs old, predominantly Caucasian male (79%) and obese with a body mass index of 29.8; similar to their pre-CADRe study BMI of 29.1. Subjects are an average of 3.2 years post CADRe Study completion or anticipated completion. Of the individual CADRe Study lifestyle components, participants were most compliant with exercise (goal ≥ 180 minutes/week): mean weekly time was 183 minutes of moderate to vigorous physical activity. Although few participants report a strict vegan dietary pattern following completion of the CADRe Study, dietary fiber intake was higher than the average U.S intake at 29 grams/day and the average fruit and vegetable intake was 9.7 servings per day. As in the CADRe Study, participants continued to have difficulty in daily performance of stress management goal of 60 minutes daily. Participants reported an average time of 154 minutes/week spent in any of the five CADRe Study techniques. Only 33% of the participants reported performance of at least 1 of the 5 stress management techniques taught in the CADRe Study on a daily basis. Table 2 provides a preliminary descriptive analysis for the major outcome variables as an aggregate sample for the initial study visit. Individual HHI scores (composite score of heart healthy behaviors) has not yet been calculated, however, at least 5 of the 7 heart healthy behaviors are being met by the aggregate sample (LDL-cholesterol < 100 mg/dL; dietary fiber intake ≥ 25

gms/day; consumption of 5 or more fruits and vegetables per day; BP < 140/90 mmHg, and regular exercise ≥ 150 min/week).

INTERIM DISCUSSION:
The persistence lifestyle change behaviors and risk factor control cannot be adequately evaluated since individual HHI scores have not yet been calculated. However, changes in each modifiable CVD risk factor (blood pressure, body composition and fitness, lipid levels and glucose) can be assessed in comparison to the final CADRe Study visit. In looking at Year 5 data only, subjects show little change in blood pressure, glucose control and HDL-cholesterol. Body anthropometrics show a mean weight gain of 6 lbs and a 5% increase in body fat despite reporting > 150 minutes per week of vigorous to moderate physical activity. Subjects also report consuming more vegetable and fruit per day than at their final CADRe study visit. Although subjects showed a decline in consumption of daily fiber, they are consuming more than the recommended > 25 grams per day. No change was seen in C-reactive protein and HDL-cholesterol. However, significant reductions in total cholesterol and LDL-cholesterol were seen at Year 5. The change in lipid values was compared in those subjects who reported an increase in cholesterol-lowering medications versus those who reported a decrease or no change in these medications. No significant difference was detected in this comparison. Quality of Life indicators were evaluated using the SF-36 Functional Health Survey. In this sample, little change was seen in the overall mental health of the sample, but a significant difference was noted in overall physical functioning at the Year 5 visit. Since new co-morbid conditions are not collected in this study, it may be difficult to interpret the change in the physical functioning of this sample. The 5-year increase in age may help explain some of the variance in this outcome variable.

Table 2: Major Outcomes Variables at Initial Study Visit

Outcome Variable (n=76)	Mean	SD
Weight (kg)	88.4	22.5
Body Mass Index (BMI)	29.8	6.3
% Body Fat (n=74)	31.2	9.8
Systolic BP (mmHg)	125.6	12.6
Diastolic BP (mmHg)	71.3	6.8
Fasting Glucose (mg/dL)	94.8	13.4
Total Cholesterol (mg/dL)	154.4	35.5
HDL Cholesterol (mg/dL)	50.2	11.4
LDL Cholesterol (mg/dL)	86.2	30.7
Triglycerides (mg/dL)	129.6	67.8
C-Reactive Protein (mg/dL) (n=74)*	0.197	0.365
Daily Dietary Fiber (gms)	28.6	14.1
Daily Fruit/Vegetable Servings	9.7	5.5
Weekly Exercise Time (minutes)	183.0	167.0
Weekly Stress Mgt Time (minutes)	154.1	166.8
Physical Composite Score	44.2	11.5
Mental Composite Score	54.1	8.2

*Two outliers (C - reactive protein > 6.00) excluded from analysis

Adverse Events: None

Task #4: Initiate "*B*etter Adherence *t*o *T*herapeutic *L*ifestyle Change *E*fforts (BATTLE) Trial".

The **B**etter **A**dherence **t**o **T**herapeutic **L**ifestyle Change **E**fforts (**BATTLE**) Trial is a two-arm, randomized, clinical trial that will determine if CIMT ultrasound images motivate adherence to a therapeutic lifestyle change (TLC) intervention program involving a Mediterranean-type diet, exercise, and group support. The study population will have CVD risk factors and significant subclinical atherosclerosis, determined by carotid ultrasound images of carotid intima-medial thickness (CIMT). All subjects will participate in the same TLC program. However, one group of subjects will receive their CIMT results at the beginning of the TLC program and the other group will receive these results after completion of the study. The primary outcome measure is the change in a composite score for adherence with the TLC intervention. Other outcomes to be studied include: adherence to individual TLC program components, change in CVD risk factors, inflammatory and diabetes-related metabolic markers, and anxiety. This study will provide new scientific evidence on the value of CIMT testing as a motivational tool for individuals seeking lifestyle change training in the setting of a cardiac prevention clinic.

Status: WRAMC HUC approved protocol on 25 April 2006 and forwarded study to CIRO for final approval. CIRO determined COL(ret) Vernalis, Henry M. Jackson Foundation employee, under AR 40-38 was ineligible to act as study PI and requested PI change to a **WRAMC-assigned** investigator. Approval received for COL Allen Taylor, Chief, WRAMC Cardiology Services, to assume the PI role and COL(ret) Marina Vernalis assume the AI role. Final study approval from CIRO was granted on 28 April 2006. The MRMC Memorandum of Deferral was received 5 Jul 06. After additional review by CIRO, the project was notified that COL(ret) Vernalis could serve as a Co-PI for the study. A Change of PI from COL(Ret) Allen Taylor to COL Randolph Modlin was approved by WRAMC DCI on 3 December 2008. All appropriate documents have been changed to reflect the new PI.

Addendum #1 incorporated the addition of a CIMT tutorial and knowledge assessment test and was approved with the initial protocol approval.

Addendum #2 was submitted to WRAMC HUC on 15 September 2006 for the following changes: COL(ret) Vernalis as Co-PI, deletion of Dr. Debra Marshall as AI, addition of Elaine Walizer, MSN, RN as AI and modification to investigator roles and responsibilities. Approval was received 5 January 07.

Protocol Addendum #3 was submitted to WRAMC DCI on 24 July 2006 and reviewed by WRAMC HUC on 26 September 2006. Approved meeting minutes outlining requested revisions were received on 2 Nov 06. Requested revisions were submitted to WRAMC DCI on 3 Nov 07 with final approval received 5 January 07. The following changes to this study are as follows:

1) Additional advertisements (simplified version of approved advertisement specifically for newspaper and electronic media)
2) Expanded version of Mediterranean Diet Short Dietary Intake Questionnaire
3) Inclusion of self-efficacy and motivation measures
4) Location change of laboratory performing Omega-3 Index

Protocol Addendum #4 was submitted to WRAMC DCI on 22 September 2007 and approved by WRAMC HUC on 10 October 2007. This addendum changes two sentences in the protocol to allow all participants to attend the weekly on site sessions not differentiated by group, and ask participants no to speak about which randomization group they have been assigned. This change will minimize the potential risk of un-blinding the research staff and study subjects.

Protocol Addendum #5 was submitted to WRAMC DCI on 3 October 2008 and approved by WRAMC HUC on 6 October 2008. The following changes to the study are as follows:

1) Change study completion date from Sept 07 to Dec 09.
2) Expand recruitment methods to include recruitment tables in the WRAMC public areas and Internal Medicine Clinic.

Study recruitment activities have expanded to include the following WRAMC locations: Cardiology Clinic, Internal Medicine and several WRAMC public areas. Flyers are posted throughout WRAMC including the numerous Hospital clinics, Hospital Lobby, Dining Facility, PX, Fitness Center and Borden Pavilion. Since November 2007, 521patients have given permission for a study team member to contact them regarding this study. Figure 1 below depicts a flow diagram of the recruitment process and current recruitment / screening sampling. Approximately 32% of "interested" patients telephonically screened were eligible to initiate the study screening process. Over 57% of those contacted (n=297) opted out of the study primarily for time commitment and travel/distance reasons. The primary reason for ineligibility on the initial telephone screen was low cardiovascular risk profile. Of the 160 subjects consented for screening, 95 subjects (59%) screened out primarily by CIMT (<75 percentile for gender/age). Of the 65 subjects eligible to make Screening Visit #2, 62 have completed this visit with 81% becoming eligible to participate in the Screening Run-In (RI) phase. To date, 40 of the 50 RI eligible subjects entered the RI phase with 38 subjects ultimately meeting all study screening criteria and invited to participate in the Main Study. In summary, 9% of patients telephonically contacted were eligible to participate in the RI phase of the study. Additionally, approximately 24% of those patients who meet initial screening criteria were randomized into the main study.

Eight RI groups (n=40) have been conducted to date with 38 subjects successfully completing the RI phase and randomizing into the Main Study. Eight Main Study Support Groups have been conducted yielding 23 study completers, 7 non-completers and 8 subjects actively engaged in the Support Group sessions. Of the 7 non-completers, 4 withdrew their consent after randomization, 1 was lost to follow-up, and 2 could not complete the study due to a non-study related adverse event.

Although complete study data is being managed by PREMIER Research and study subject group assignment is not known the study research team, some preliminary analysis has been conducted on site.

Of the 38 randomized subjects, the mean age is 55.9 ± 10.3 with a range of 34 to 73 years old, 58% female, 60% were from minority groups and 16% have Type 2 diabetes. Of the randomized subjects, 24% were active duty, 29% were retired from the military services, and 47% were eligible family members. At baseline, randomized subjects were obese (BMI = 30.7 ± 5.6; weight =191 ± 44 lbs; % Body Fat = 35.9 $\underline{\pm}$ 8) with 84% of subjects having a BMI $\underline{\geq}$ 25.

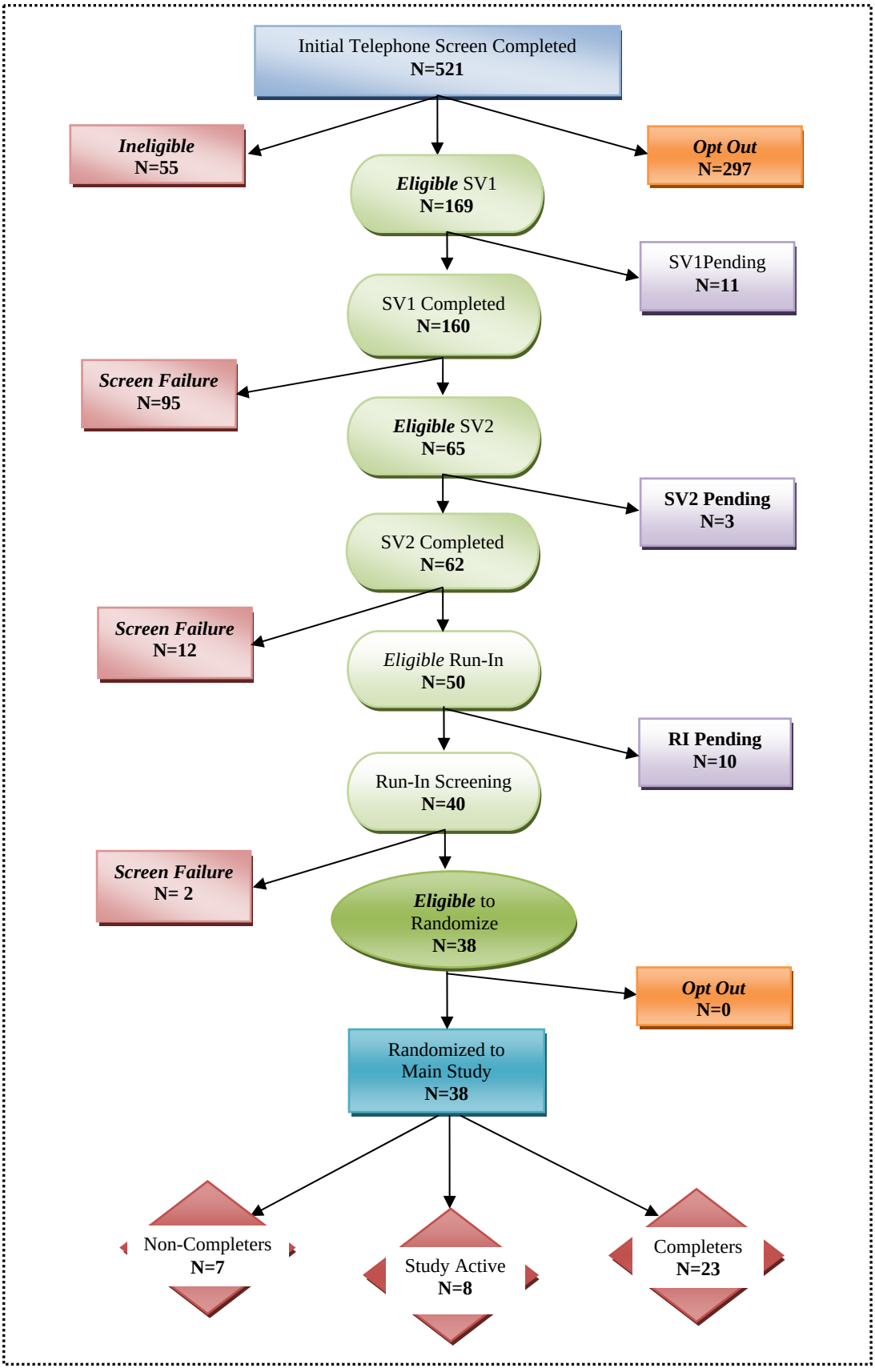

Initial Telephone Screen Completed
N=521
Ineligible
N=55
Opt Out
N=297
Eligible SV1
N=169
SV1Pending
N=11
SV1 Completed
N=160
Screen Failure
N=95
Eligible SV2
N=65
SV2 Pending
N=3
SV2 Completed
N=62
Screen Failure
N=12
Eligible Run-In
N=50
RI Pending
N=10
Run-In Screening
N=40
Screen Failure
N= 2
Eligible to Randomize
N=38
Opt Out
N=0
Randomized to Main Study
N=38
Non-Completers
N=7
Study Active
N=8
Completers
N=23

Figure 1.

Table 3 depicts the change in select outcome variables to date between baseline and study completion in the 22 subjects who have provided study completion data. Preliminary data analysis on these completers, comparing study completion to baseline, was conducted using the paired *t-test*. Measures of obesity including weight and BMI declined 5% and percent of body fat was reduced by 5%. Additionally, a 6% reduction in waist circumference and an 8% reduction in abdominal sagittal diameter were seen. Serum glucose was reduced by 8%, triglycerides were lowered by 16% and fasting insulin was reduced by 20%. Although not statistical significant, levels of total cholesterol were reduced by 5%, LDL-cholesterol decreased by 4% and C-reactive protein was decreased by 30%. Despite these positive changes, a 7% reduction in HDL-cholesterol was seen. Overall, this data further supports participation in a lifestyle modification program which includes education and frequent monitoring does result in substantial cardiovascular risk factor improvements. Some of these changes rival what is observed with pharmacological treatment.

Table 3. Select Outcome Variables in Study Completers (n=22)

	Baseline	Study Completion	Change	P
Body Composition				
Weight (kg)	87.7 ± 19.3	82.9 ± 16.9	-4.9 ± 4.2	<0.001
BMI (kg/m^2)	30.5 ± 5.3	28.9 ± 4.7	-1.7 ± 1.3	<0.001
% Body Fat	34.7 ± 8.4	33.0 ± 8.7	-1.7 ± 2.2	0.001
Sagittal Diameter (cm)	23.9 ± 3.7	22.0 ± 3.0	-1.9 ± 1.8	<0.001
Waist Circumference (cm)	99.7 ± 12.7	93.6 ± 11.5	-6.1 ± 4.9	<0.001
Laboratory (mg/dL)				
Glucose (mg/dL)	101.2 ± 23.4	91.1 ± 13.0	-10.1 ± 20.0	0.028
Total Cholesterol (mg/dL)	177.4 ± 34.6	168.9 ± 36.9	-8.5 ± 20.7	0.068
LDL-Cholesterol(mg/dL)	105.0 ± 29.8	100.9 ± 33.2	-4.1 ± 19.0	0.328
HDL-Cholesterol(mg/dL)	51.7 ± 13.9	47.5 ± 9.9	-4.3 ± 7.2	0.011
Triglycerides (mg/dL)	134.7 ± 68.9	101.3 ± 43.6	-33.5 ± 54.2	0.009
C-reactive protein (mg/dL)	0.333 ± 0.366	0.275 ± 0.397	-0.057 ± 0.214	0.225
Insulin (uIU/mL)	14.3 ± 11.8	10.4 ± 7.2	-3.9 ± 6.5	0.012

Values are mean ± SD.

Adverse Events: There are 3 serious and 5 non-serious adverse events (AEs) reported since enrollment began. The serious AEs were unrelated to study participation; however, 2 of the 5 non-serious AEs are possibly related to participation in this study. All adverse events have been reported to the WRAMC DCI and HJF. No changes have been requested to the study informed consent documents.

Task #5: Ongoing enrollment to Dr. Dean Ornish Program for Reversing Heart Disease protocol.

Status: Study is currently ongoing.

Background: The Dr. Dean Ornish Program for Reversing Heart Disease

The Ornish Program, an intensive lifestyle modification program, was established in January 2000 at Windber Medical Center (WMC). The objective of the study at WMC is to determine the one-year efficacy of the intensive lifestyle modification program with a focus on diet, exercise, stress management, and group support for improving the clinical status of patients with moderate to severe coronary artery disease or risk factors that would promote coronary heart disease (CHD). Outcome measures include: (1) biochemical/anthropometric measures: blood lipids, glucose, glycosylated hemoglobin, blood pressure, weight, body composition, body mass

index (BMI), and exercise capacity achieved on exercise stress testing; (2) psychometric test scores: depression, hostility, preferred support, perceived stress, and quality of life; and (3) adherence outcome measures: retention and attrition rates, attendance, and overall and individual adherence scores for stress management (7 days a week for a total of 420 minutes), moderate aerobic exercise (180 minutes per week), and diet (low-fat vegan diet).

Subject Enrollment and Demographics

Subject enrollment to date is 422 participants including 25 cohorts and 4 retreats. There are 10 subjects who continue to actively participate in the year-long program, 339 have graduated, and 83 have discontinued participation (24% dropout rate). Demographic characteristics of participants are: average age of 65.2 years, 53% are female, 33% are veterans or the spouse of a veteran, and 41% have diagnosed coronary heart disease.

Subject Adherence

Experience throughout the study over the past seven years has shown that subjects are able to make and maintain comprehensive changes in nutrition and lifestyle over a minimum period of one year (Table 4). During their entire year of participation, subjects in the WMC Ornish Program are required to complete a Personal Awareness Log (PAL form) weekly, which includes daily documentation of nutritional intake, stress management, exercise, and group support. PAL forms are reviewed weekly by the clinical staff. Weekly staff meetings are held to discuss each subject's medical status and adherence, and clinical staff provides feedback to assist and encourage subjects to increase adherence to the defined guidelines as required.

Table 4. Adherence to dietary, exercise, stress management, and group support guidelines over 12-week and one-year periods for participants in the Ornish Program for Reversing Heart Disease at Windber Medical Center*

Guideline	Compliance goal (%)	12 Weeks		1 Year	
		Average % compliance (n=259)	% of goal	Average % compliance (n=259)	% of goal
Diet (% compliance)†	100	94.63	94.63	95.33	95.33
Stress management (% compliance)	100	98.49	98.49	99.20	99.20
Exercise (% compliance)	100	120.36	120.36	114.46	114.46
Group support (% attendance)	80	96.60	120.75	88.02	110.02
Total adherence score‡	--	--	108.55	--	104.75

* Includes the most recent relevant data from Cohorts #1 through #25 as of December 8, 2008; excludes subjects who discontinued participation in the program.

† Diet - compliance measured as a percentage of the recommended diet goals actually achieved; stress management - compliance is a percentage of the recommended level (one hour/day, seven days/week) actually attained; exercise - compliance is the percentage of the recommended level (180 min/week) actually achieved; group support - compliance is the percentage of sessions attended.

‡ Calculated as the average percentage of lifestyle changes achieved by participants at the 12-week and one-year examinations.

Outcome Data

Participants in the Dr. Dean Ornish Program at Windber Medical Center have achieved significant improvement in levels of virtually all of the measured coronary artery disease (CAD) risk factors over the initial 12-week period (Table 5A). Measures of obesity including weight and BMI declined ~7%, levels of total cholesterol were reduced by nearly 13%, blood pressure

dropped ~9%, measures of physical fitness increased more than 26%, and levels of depression decreased approximately 47%. These data demonstrate that lifestyle change programs may be important for primary prevention in individuals with diagnosed CAD and those at increased risk of disease. Results from the end of the year examination are shown in Table 5B. Over the course of one year, weight and BMI decreased ~9%, diastolic blood pressure decreased ~7%, measures of physical fitness increased 25%, and levels of depression decreased nearly 50%.

Table 5A. Change in Outcome Variables after 12 weeks for 259 Participants in the Lifestyle Change Program for Heart Disease Reversal

Category / Metrics	N	Mean Baseline (SD)	Mean Week 12 (SD)	Mean Change	P Value
Weight (lbs.)	259	204.49 (45.4)	190.92 (40.7)	-13.6	<0.00001
Body Mass Index	254	32.30 (6.9)	30.20 (6.1)	-2.1	<0.00001
Total Cholesterol (mg/dl)	259	193.37 (42.4)	167.80 (36.8)	-25.6	<0.00001
High Density Lipids (mg/dl)	259	46.82 (12.5)	40.36 (9.5)	-6.5	<0.00001
Low Density Lipids (mg/dl)	248	110.44 (35.2)	94.09 (29.7)	-16.3	<0.00001
Triglycerides (mg/dl)	259	180.51 (94.7)	166.06 (79.0)	-14.5	<0.01
Systolic Blood Pressure	259	136.14 (18.4)	123.97 (15.1)	-12.2	<0.00001
Diastolic Blood Pressure	259	80.51 (10.4)	72.98 (8.8)	-7.5	<0.00001
Exercise Capacity (min.) [BRUCE]	256	7.00 (2.6)	8.82 (2.6)	1.82	<0.00001
Oxygen Capacity [METS]	255	8.42 (2.6)	10.21 (2.8)	1.79	<0.00001
Depression Scale [CES-D]	259	11.68 (10.0)	6.22 (6.4)	-5.5	<0.00001
Hostility Scale [Cook-Medley]	259	8.12 (4.7)	6.15 (4.3)	-2.0	<0.00001
Daily Total Fat (grams)	250	63.97 (38.2)	18.41 (6.1)	-45.6	<0.00001
Daily Saturated Fat (grams)	250	19.73 (13.5)	3.20 (1.4)	-16.5	<0.00001
% Daily Caloric Fat Intake	250	28.05 (9.9)	10.56 (2.9)	-17.5	<0.00001

Table 5B. Change in Outcome Variables after one year for 246 Participants in the Lifestyle Change Program for Heart Disease Reversal

Category / Metrics	N	Mean Baseline (SD)	Mean Year 1 (SD)	Mean Change	P Value
Weight (lbs.)	246	203.25 (43.7)	185.28 (39.4)	-18.0	<0.00001
Body Mass Index	233	32.35 (6.7)	29.48 (6.0)	-2.9	<0.00001
Total Cholesterol (mg/dl)	246	193.06 (42.1)	181.28 (38.9)	-11.7	<0.00001
High Density Lipids (mg/dl)	246	46.90 (12.5)	45.69 (12.7)	-1.2	0.0656
Low Density Lipids (mg/dl)	234	109.96 (34.6)	102.03 (30.8)	-7.9	<0.0001
Triglycerides (mg/dl)	246	181.57 (95.5)	170.56 (87.9)	-11.0	<0.05
Systolic Blood Pressure	246	136.07 (18.4)	127.48 (18.6)	-8.6	<0.00001
Diastolic Blood Pressure	246	80.50 (10.4)	74.86 (9.7)	-5.6	<0.00001
Exercise Capacity (min.) [BRUCE]	233	7.06 (2.6)	9.17 (2.9)	2.10	<0.00001
Oxygen Capacity [METS]	233	8.44 (2.6)	10.55 (2.9)	2.11	<0.00001
Depression Scale [CES-D]	242	11.84 (10.1)	6.06 (6.1)	-5.8	<0.00001
Hostility Scale [Cook-Medley]	242	8.16 (4.8)	5.70 (4.1)	-2.5	<0.00001
Daily Total Fat (grams)	210	62.92 (36.9)	22.39 (9.5)	-40.5	<0.00001
Daily Saturated Fat (grams)	210	19.65 (13.5)	4.23 (2.5)	-15.4	<0.00001
% Daily Caloric Fat Intake	224	27.75 (9.8)	11.59 (3.8)	-16.2	<0.00001

In subjects matched for age, gender, and disease status who did not participate in the Ornish lifestyle change program, most risk factors did not show significant changes after 12 weeks in the Program (Table 6A). BMI and systolic blood pressure decreased, while HDL and physical fitness measures increased.

Table 6A. Change in Outcome Variables after 12 Weeks for 114 Subjects Matched for Age, Gender, and Disease Status Who Did Not Participate in a Lifestyle Change Program

Category / Metrics	N	Mean Baseline (SD)	Mean Week 12 (SD)	Mean Change	P Value
Weight (lbs.)	114	182.58 (35.1)	181.98 (35.3)	-0.6	0.2010
Body Mass Index	95	28.35 (4.0)	28.03 (4.2)	-0.3	<0.05
Total Cholesterol (mg/dl)	114	191.08 (43.9)	191.03 (43.3)	-0.1	0.9860
High Density Lipids (mg/dl)	114	49.95 (13.1)	51.87 (13.0)	1.9	<0.01
Low Density Lipids (mg/dl)	108	111.94 (34.7)	108.52 (34.4)	-3.4	0.1881
Triglycerides (mg/dl)	114	148.11 (102.9)	158.54 (128.8)	10.4	0.2363
Systolic Blood Pressure	112	134.75 (17.8)	127.71 (16.8)	-7.0	<0.001
Diastolic Blood Pressure	112	79.04 (9.7)	77.46 (8.3)	-1.6	0.0735
Exercise Capacity (min.) [BRUCE]	109	9.65 (3.0)	9.93 (3.0)	0.28	<0.01
Oxygen Capacity [METS]	109	10.97 (2.7)	11.38 (2.6)	0.41	<0.01
Depression Scale [CES-D]	113	6.49 (7.2)	5.75 (6.4)	-0.7	0.1955
Hostility Scale [Cook-Medley]	113	7.66 (4.8)	7.73 (4.9)	0.1	0.8195
Daily Total Fat (grams)	27	69.03 (32.5)	65.05 (28.5)	-4.0	0.3439
Daily Saturated Fat (grams)	27	21.76 (10.7)	20.73 (7.7)	-1.0	0.4566
% Daily Caloric Fat Intake	27	33.22 (9.8)	32.85 (6.6)	-0.4	0.7412

Likewise, most outcome variables did not change significantly over the course of one year in control subjects who did not participate in the lifestyle change program (Table 6B). Only HDL and systolic BP showed significant changes. These changes may be attributable to being a participant in the control group – people become more aware and may begin trying to improve their risk profile.

Table 6B. Change in Outcome Variables after One Year for 96 Subjects Matched for Age, Gender, and Disease Status Who Did Not Participate in a Lifestyle Change Program

Category / Metrics	N	Mean Baseline (SD)	Mean Year 1 (SD)	Mean Change	P Value*
Weight (lbs.)	96	181.87 (33.0	182.13 (35.0)	0.3	0.7095
Body Mass Index	78	28.48 (3.8)	28.51 (3.9)	0.0	0.7996
Total Cholesterol (mg/dl)	93	191.30 (43.8)	192.13 (44.1)	0.8	0.8135
High Density Lipids (mg/dl)	93	50.61 (13.3)	48.03 (13.3)	-2.6	<0.001
Low Density Lipids (mg/dl)	89	112.37 (35.3)	112.87 (36.2)	0.5	0.8715
Triglycerides (mg/dl)	93	146.73 (100.5)	153.27 (93.6)	6.5	0.3329
Systolic Blood Pressure	92	134.52 (17.5)	126.58 (14.5)	-7.9	<0.00001
Diastolic Blood Pressure	92	78.93 (9.5)	77.70 (8.2)	-1.2	0.2181
Exercise Capacity (min.) [BRUCE]	86	10.18 (2.8)	10.24 (2.8)	0.06	0.6422
Oxygen Capacity [METS]	86	11.43 (2.5)	11.61 (2.6)	0.18	0.2825
Depression Scale [CES-D]	97	6.33 (7.3)	6.54 (7.1)	0.2	0.7724
Hostility Scale [Cook-Medley]	97	7. 69 (4.8)	7.98 (4.9)	0.3	0.3966

Psychometric Measures

The citation for our published manuscript discussing psychometric measures is:
Vizza J, Neatrour DM, Felton PM, Ellsworth DL. Improvement in psychosocial functioning during an intensive cardiovascular lifestyle modification program. J Cardiopulm Rehabil Prev 2007:27:376-383.

Adverse Events

All adverse events are submitted and adjudicated by the Windber Medical Center Institutional Review Board and TATRC after review by both the Principal Investigator and Medical Monitor. There have been 103 adverse events over the course of the study, of which 66 were serious events and 37 were considered non-serious. A serious event is defined as occurring at any dose or intervention level that results in any of the following outcomes: (1) results in death, (2) a threat to life, (3) inpatient hospitalization or prolongation of existing hospitalization, (4) persistent or significant disability or incapacity, (5) causes cancer, (6) is an overdose, or (7) any medical event that requires treatment to prevent one of the medical outcomes listed above. Therefore, 56 events were considered serious due to inpatient hospitalizations. There were 69 non-cardiac and 34 cardiac adverse events. No deaths occurred and none of these adverse events were deemed to be study related.

Task #6: Ongoing enrollment to Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal, Sub-Study for Subjects in the Dr. Dean Ornish Program protocol.

Status: Enrollment to the global profiling study is ongoing. Enrollment in the sub-study was closed as of July 27, 2007.

1. Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal. This study is characterizing: 1) longitudinal changes in gene and protein expression in blood during an intensive lifestyle intervention; 2) associations of gene/protein expression profiles and single nucleotide polymorphisms (SNPs) with changes in quantitative CHD risk factors/CHD status during the intervention; and 3) gene/protein expression profiles and SNPs predictive of significant differences among individuals in patterns of change in CHD risk factors and CHD phenotypes during the lifestyle change program. This research has the potential to provide a global view of molecular changes associated with lifestyle modifications designed to reverse CHD and improve our understanding of molecular events crucial to CHD development.

Collaborators and external relationships for this project include Marina Vernalis, Debra Marshall, Elaine Walizer at Walter Reed Army Medical Center, and LipoScience, Inc., the Department of Endocrinology, University of Maryland, Baltimore, Johns Hopkins University, and Josef Machac, MD, at Mt. Sinai Medical Center.

Subject Enrollment and Demographics

Subject enrollment to date is 374. There are 166 participants taking part in the lifestyle change program and 140 subjects enrolled thus far serving as the control group. Demographic characteristics of the control group are: average age of 62.3years, 51% are female, 29% are veterans or the spouse of a veteran, and 34% have diagnosed coronary heart disease.

Sample Collection

From July 1, 2007 to November 30, 2008, there were 19 examinations and blood draws for Ornish Program and control group participants. During the year, approximately 4,478 aliquots of biological material were collected from 168 subject visits as summarized in the following table:

Cohorts and research group examinations	19
Patient visits	168
PAXGene tubes	197
Plasma samples	3,494
DNA and RBC samples	787
Total aliquots	4,478

NMR Lipid Panel

Participants continued to experience clinically-important improvements in a number of components of the NMR lipid panel, which gives detailed information on particle size and number for VLDL, HDL, and LDL (Table 7A). These data are important because several studies have linked selected variables, such as LDL particle number (LDLP) more strongly to CAD risk than LDL-cholesterol. Recommended LDLP goals are <1000 nmol/L (<20th percentile) for high risk patients and <1300 nmol/L (<50th percentile) for moderately high-risk patients. As previously observed for selected biochemical variables, some of the NMR lipid panel variables do not maintain early improvement levels and show some regression toward baseline values by the end of one year (Table 7B).

Table 7A. Change in NMR Lipids After 12 Weeks in 138 Participants in the Lifestyle Change Program for CAD Reversal

Category	N	Mean Baseline (SD)	Mean Week 12 (SD)	Mean Change	P
VLDL Particle Concentrations (nmol/L)					
VLDL Particles (total) [VLDLP]	138	87.51 (39.5)	97.59 (43.8)	10.1	<0.001
Large VLDL/Chylomicrons [VL]	138	8.36 (7.7)	5.39 (6.1)	-3.0	<0.00001
Medium VLDL [VM]	138	39.34 (23.7)	51.95 (31.8)	12.6	<0.00001
Small VLDL [VS]	138	39.81 (19.3)	40.26 (18.2)	0.4	0.7994
LDL Particle Concentrations (nmol/L)					
LDL Particles (total) [LDLP]	138	1391.38 (432.2)	1225.17 (427.1)	-166.2	<0.00001
IDL	138	72.48 (51.4)	46.21 (41.9)	-26.3	<0.00001
Large LDL [LL]	138	244.86 (203.8)	209.80 (148.3)	-35.1	<0.01
Small LDL (total) [LS]	138	1074.07 (437.5)	969.18 (414.7)	-104.9	<0.001
Medium Small LDL [LMS]	138	225.26 (88.9)	200.24 (87.1)	-25.0	<0.0001
Very Small LDL [LVS]	138	848.74 (352.7)	768.96 (330.4)	-79.8	<0.01
HDL Particle Concentrations (µmol/L)					
HDL Particles (total) [HDLP]	138	32.66 (5.7)	29.48 (4.9)	-3.2	<0.00001
Large HDL [HL]	138	4.93 (3.2)	4.49 (2.4)	-0.4	<0.01
Medium HDL [HM]	138	5.55 (4.3)	4.06 (3.8)	-1.5	<0.00001
Small HDL [HS]	138	22.18 (6.1)	20.93 (4.7)	-1.2	<0.01
Mean Particle Sizes (nm diameter)					
VLDL Size [VZ]	138	56.23 (10.2)	48.82 (8.3)	-7.4	<0.00001
LDL Size [LZ]	138	20.25 (0.7)	20.26 (0.6)	0.0	0.8711
HDL Size [HZ]	138	8.67 (0.3)	8.70 (0.3)	0.0	0.0930

Calculated Lipids (mg/dL)					
Triglycerides [NTG]	138	187.54 (96.5)	167.92 (81.3)	-19.6	<0.01
VLDL-TG [NVTG]	138	149.99 (95.1)	136.64 (80.1)	-13.4	<0.05
HDL-C [NHDLC]	138	44.30 (11.4)	39.84 (8.6)	-4.5	<0.00001

Table 7B. Change in Selected NMR Lipids After One Year in 88 Participants in the Lifestyle Change Program for CAD Reversal

Category	N	Mean Baseline (SD)	Mean Year 1 (SD)	Mean Change	P
VLDL Particle Concentrations (nmol/L)					
VLDL Particles (total) [VLDLP]	88	86.46 (41.3)	88.19 (44.6)	1.7	0.6828
Large VLDL/Chylomicrons [VL]	88	7.97 (6.5)	5.42 (7.0)	-2.6	<0.001
Medium VLDL [VM]	88	37.71 (24.7)	42.89 (29.5)	5.2	0.0599
Small VLDL [VS]	88	40.77 (20.5)	39.89 (20.9)	-0.9	0.7155
LDL Particle Concentrations (nmol/L)					
LDL Particles (total) [LDLP]	88	1436.89 (454.2)	1301.69 (426.2)	-135.2	<0.001
IDL	88	78.22 (55.7)	55.47 (51.5)	-22.8	<0.001
Large LDL [LL]	88	256.25 (221.0)	262.65 (174.9)	6.4	0.7076
Small LDL (total) [LS]	88	1102.49 (468.8)	983.52 (452.6)	-119.0	<0.01
Medium Small LDL [LMS]	88	229.43 (96.8)	205.94 (101.1)	-23.5	<0.01
Very Small LDL [LVS]	88	872.97 (376.9)	777.61 (355.1)	-95.4	<0.01
HDL Particle Concentrations (µmol/L)					
HDL Particles (total) [HDLP]	88	32.61 (5.4)	32.50 (5.7)	-0.1	0.8491
Large HDL [HL]	88	5.05 (3.3)	5.29 (2.9)	0.2	0.3687
Medium HDL [HM]	88	5.37 (4.5)	5.14 (5.4)	-0.2	0.6439
Small HDL [HS]	88	22.19 (6.1)	22.07 (6.9)	-0.1	0.8424
Mean Particle Sizes (nm diameter)					
VLDL Size [VZ]	87	55.87 (10.1)	49.77 (9.3)	-6.1	<0.00001
LDL Size [LZ]	88	20.27 (0.8)	20.43 (0.7)	0.2	<0.01
HDL Size [HZ]	88	8.68 (0.3)	8.74 (0.4)	0.1	0.0516
Calculated Lipids (mg/dL)					
Triglycerides [NTG]	88	183.61 (91.0)	160.03 (93.2)	-23.6	<0.01
VLDL-TG [NVTG]	88	144.70 (88.7)	124.95 (89.7)	-19.8	<0.05
HDL-C [NHDLC]	88	44.51 (11.5)	45.06 (9.8)	0.5	0.5926

In contrast, control subjects who did not participate in the Lifestyle Change Program for CAD Reversal showed few significant changes in NMR lipid values after 12 weeks (Table 8A) and one year (Table 8B). Of note, for NMR measures that do show a significant change in control subjects, the magnitude of these changes is usually a small percentage of the baseline value and thus are not likely to be clinically significant. Because these individuals were not participating in any intervention to alter lipid levels, we would expect no significant changes in NMR lipids. This comparison, does however, demonstrate the impact of the Lifestyle Change Program in altering important measures of CAD risk.

Table 8A. Change in NMR Lipids After 12 Weeks in 101 Subjects who did not Participate in the Lifestyle Change Program for CAD Reversal

Category	N	Mean Baseline (SD)	Mean Week 12 (SD)	Mean Change	P
VLDL Particle Concentrations (nmol/L)					
VLDL Particles (total) [VLDLP]	101	81.81 (47.2)	79.86 (44.0)	-2.0	0.5316
Large VLDL/Chylomicrons [VL]	101	4.89 (7.2)	5.93 (7.8)	1.0	<0.05
Medium VLDL [VM]	101	37.79 (29.4)	37.45 (25.2)	-0.3	0.8755
Small VLDL [VS]	101	39.14 (20.2)	36.48 (21.2)	-2.7	0.1190
LDL Particle Concentrations (nmol/L)					
LDL Particles (total) [LDLP]	101	1260.16 (395.9)	1230.98 (425.2)	-29.2	0.3326
IDL	101	49.00 (43.0)	50.17 (53.6)	1.2	0.8053
Large LDL [LL]	101	329.44 (207.6)	320.67 (195.7)	-8.8	0.4478
Small LDL (total) [LS]	101	881.66 (445.9)	860.14 (448.6)	-21.5	0.4977
Medium Small LDL [LMS]	101	182.54 (92.5)	179.56 (87.5)	-3.0	0.6547
Very Small LDL [LVS]	101	699.14 (357.3)	680.59 (363.2)	-18.5	0.4721
HDL Particle Concentrations (µmol/L)					
HDL Particles (total) [HDLP]	101	34.53 (6.5)	35.22 (5.9)	0.7	0.0541
Large HDL [HL]	101	6.37 (3.4)	6.74 (3.4)	0.4	<0.05
Medium HDL [HM]	101	5.06 (4.7)	5.23 (5.2)	0.2	0.5838
Small HDL [HS]	101	23.10 (7.0)	23.25 (7.1)	0.2	0.7175
Mean Particle Sizes (nm diameter)					
VLDL Size [VZ]	101	50.69 (8.6)	52.34 (8.2)	1.7	<0.05
LDL Size [LZ]	101	20.68 (0.8)	20.70 (0.8)	0.0	0.6821
HDL Size [HZ]	101	8.85 (0.4)	8.88 (0.4)	0.0	0.1536
Calculated Lipids (mg/dL)					
Triglycerides [NTG]	101	151.23 (99.7)	158.05 (101.6)	6.8	0.2969
VLDL-TG [NVTG]	101	115.45 (97.8)	122.28 (98.8)	6.8	0.2826
HDL-C [NHDLC]	101	50.10 (12.6)	51.64 (12.1)	1.5	<0.05

Table 8B. Change in NMR Lipids After One Year in 69 Subjects who did not Participate in the Lifestyle Change Program for CAD Reversal

Category	N	Mean Baseline (SD)	Mean Week 12 (SD)	Mean Change	P
VLDL Particle Concentrations (nmol/L)					
VLDL Particles (total) [VLDLP]	69	78.63 (45.8)	78.24 (46.1)	-0.4	0.9190
Large VLDL/Chylomicrons [VL]	69	3.84 (4.9)	4.14 (5.3)	0.3	0.6315
Medium VLDL [VM]	69	35.67 (29.0)	35.61 (29.4)	-0.1	0.9854
Small VLDL [VS]	69	39.11 (19.2)	38.48 (19.8)	-0.6	0.7652
LDL Particle Concentrations (nmol/L)					
LDL Particles (total) [LDLP]	69	1241.35 (405.0)	1225.64 (412.1)	-15.7	0.7024
IDL	69	48.39 (43.9)	44.90 (46.5)	-3.5	0.5171
Large LDL [LL]	69	338.32 (195.7)	339.65 (199.1)	1.3	0.9435
Small LDL (total) [LS]	69	854.52 (434.8)	841.01 (450.8)	-13.5	0.7743
Medium Small LDL [LMS]	69	177.94 (89.3)	178.09 (93.4)	0.1	0.9885
Very Small LDL [LVS]	69	676.61 (350.2)	662.90 (359.6)	-13.7	0.7154
HDL Particle Concentrations (µmol/L)					
HDL Particles (total) [HDLP]	69	34.58 (7.1)	35.86 (7.0)	1.3	<0.05
Large HDL [HL]	69	6.58 (3.3)	6.73 (3.5)	0.2	0.5370
Medium HDL [HM]	69	5.01 (4.5)	4.83 (4.7)	-0.2	0.6999
Small HDL [HS]	69	23.00 (7.3)	24.30 (7.2)	1.3	<0.05
Mean Particle Sizes (nm diameter)					
VLDL Size [VZ]	69	50.44 (8.9)	50.75 (7.8)	0.3	0.7776
LDL Size [LZ]	69	20.74 (0.8)	20.78 (0.8)	0.0	0.6407
HDL Size [HZ]	69	8.87 (0.4)	8.85 (0.4)	0.0	0.4006
Calculated Lipids (mg/dL)					
Triglycerides [NTG]	69	141.26 (89.1)	141.23 (86.6)	0.0	0.9972
VLDL-TG [NVTG]	69	105.51 (86.1)	105.70 (83.1)	0.2	0.9814
HDL-C [NHDLC]	69	50.58 (12.7)	52.07 (13.7)	1.5	0.1047

A comparison of changes in atherogenic NMR lipoprotein variables in Ornish participants compared to other lifestyle and pharmacologic interventions is presented below in Table 8C.

Table 8C. Response of LDL particle number and LDL size to different clinical interventions

Lipoprotein [a]	Trial/Center	Intervention	Daily dosage (mg)	Mean follow-up (weeks)	Baseline value ± SD	Change (%)
LDLP (nmol/L)	VA-HIT [b]	gemfibrozil	1200	~30	1352 ± 316	-4.6
	JUSMH [c]	bezafibrate	400	4	1722 ± 629	-4.6
	UMCP [m]	exercise training	---	24	1436 ± 42 [g]	-7.0
	Ornish Program	healthy lifestyle	---	52	1437 ± 454	-9.4
	MC [l]	pioglitazone	30	19	1420 ± 74 [g]	-10.6
	JUSMH [d]	fenofibrate	200	8	1567 ± 606	-10.9
	VCU [e]	niacin IR	3000	12	2561 ± 81 [g]	-14.1
	TJU [h]	niacin ER [i]	1000	12	1993	-15.0
	MC [l]	diet & exercise	---	19	1216 ± 55 [g]	-18.8
	TJU [h]	niacin ER [i]	2000	12	2048	-23.0
	PLAC-1 [j]	pravastatin	20-40	~26	1908 ± 304	-24.0
	PLAC-1 [k]	pravastatin	40	~26	1918 ± 292	-25.5
	VCU [e]	atorvastatin	10	12	2562 ± 77 [g]	-31.4
LZ (nm)	PLAC-1 [j]	pravastatin	20-40	~26	20.7 ± 0.5	+0.3
	PLAC-1 [k]	pravastatin	40	~26	20.7 ± 0.4	+0.5
	UMCP [m]	exercise training	---	24	21 ± 0.1 [g]	+1.0
	Ornish Program	healthy lifestyle	---	52	20.3 ± 0.8	+1.0
	MC [l]	diet & exercise	---	19	20.6 ± 0.2 [g]	+1.5
	VCU [e]	atorvastatin	10	12	19.8 ± 0.1 [g]	+1.5
	TJU [h]	niacin ER [i]	1000	12	20.0	+2.0
	TJU [h]	niacin ER [i]	2000	12	21.0	+2.0
	MC [l]	pioglitazone	30	19	20.3 ± 0.2 [g]	+2.0
	VCU [e]	niacin IR	3000	12	19.9 ± 0.1 [g]	+2.5
	VA-HIT [b]	gemfibrozil	1200	~30	20.4 ± 0.8	+2.5
	JUSMH [d]	fenofibrate	200	8	19.7 ± 0.8	+3.8
	JUSMH [c]	bezafibrate	400	4	19.9 ± 1.0	+3.9

a: determined by NMR spectroscopy, b: Veterans Affairs High-Density Lipoprotein Intervention Trial – 515 men with known coronary heart disease, c: Jikei University School of Medicine Hospital – hypertriglyceridemic men (22) and women (2), d: Jikei University School of Medicine Hospital – 20 hypertriglyceridemic men, e: Virginia Commonwealth University – 53 (atorvastatin) or 48 (niacin) men and women with atherogenic dyslipidemia, treatment followed a 6-week lead-in period on a National Cholesterol Education Program Step One diet, f: immediate release (IR), g: values are mean $\pm$ SE, h: Thomas Jefferson University – 21 (1000 mg) or 20 (2000 mg) men and women with primary hypercholesterolemia, treatment followed an 8-week lead-in phase on an American Heart Association Step One diet, i: extended release (ER), j: Pravastatin Limitation of Atherosclerosis in the Coronary Arteries trial – 154 men and women with coronary heart disease, k: Pravastatin Limitation of Atherosclerosis in the Coronary Arteries trial – 130 men and women with coronary heart disease, l: Mayo Clinic – non-diabetic insulin-resistant men (18) and women (19), patients consumed an isocaloric diet for one week prior to baseline and follow-up, m: University of Maryland, College Park – sedentary healthy men (42) and women (58), subjects followed the American Heart Association Dietary Guidelines for the General Population prior to and throughout exercise training.

Inflammatory Markers

Assays are being conducted for a panel of five inflammatory markers, potentially important in CAD development and/or reversal: insulin, leptin, C-reactive protein, interleukin-6, and interleukin-8. Results are shown in Tables 9A-9B. Insulin, CRP, and leptin levels decrease significantly in Ornish participants after 12 weeks and one year. All changes would be considered improvements in CAD risk profiles.

Table 9A. Change in Inflammatory Markers After 12 Weeks in 89 Ornish Program Participants

Category	N	Mean Baseline (SD)	Mean 12 Week (SD)	Mean Change	P
Insulin [uU/ml]	88	19.19 (10.9)	15.77 (9.4)	-3.421	<0.01
HS-CRP [ug/ml	89	4.59 (6.1)	3.44 (4.0)	-1.150	<0.05
Leptin [ng/ml]	89	25.84 (19.2)	16.17 (12.2)	-9.671	<0.00001
IL-6 [pg/ml]	88	3.20 (3.2)	3.38 (3.3)	0.180	0.6755
IL-8 [pg/ml]	86	13.97 (3.0)	14.40 (3.8)	0.430	0.2500

Table 9B. Change in Inflammatory Markers After One Year in 46 Ornish Program Participants

Category	N	Mean Baseline (SD)	Mean 12 Week (SD)	Mean Change	P
Insulin [uU/ml]	46	19.11 (11.8)	15.28 (9.7)	-3.831	<0.01
HS-CRP [ug/ml	46	5.56 (7.6)	2.73 (3.2)	-2.835	<0.01
Leptin [ng/ml]	46	24.31 (19.5)	16.36 (13.8)	-7.942	<0.001
IL-6 [pg/ml]	45	3.66 (4.1)	4.86 (9.6)	1.203	0.4294
IL-8 [pg/ml]	45	13.63 (3.0)	14.10 (4.1)	0.463	0.4850

In control participants who did not participate in the lifestyle change program, only leptin levels decreased significantly at 12 weeks, but showed a significant increase by the end of one year (Tables 9C-9D). As observed above, we do not expect to see significant changes in CAD risk factors in control participants. The detrimental change (increase) in leptin may represent one component of a deteriorating CVD risk profile in individuals who are not practicing a healthy lifestyle.

Table 9C. Change in Inflammatory Markers After 12 Weeks in Control Participants

Category	N	Mean Baseline (SD)	Mean 12 Week (SD)	Mean Change	P
Insulin [uU/ml]	81	15.55 (8.8)	15.72 (7.5)	0.168	0.8600
HS-CRP [ug/ml	82	2.86 (3.4)	2.62 (3.1)	-0.237	0.5673
Leptin [ng/ml]	82	17.40 (12.3)	15.02 (10.4)	-2.381	<0.001
IL-6 [pg/ml]	82	1.87 (1.2)	1.95 (1.3)	0.076	0.6573
IL-8 [pg/ml]	75	13.06 (3.6)	13.65 (2.7)	0.590	0.1313

Table 9D. Change in Inflammatory Markers After One Year in Control Participants

Category	N	Mean Baseline (SD)	Mean 12 Week (SD)	Mean Change	P
Insulin [uU/ml]	67	15.15 (7.8)	15.49 (7.8)	0.346	0.6085
HS-CRP [ug/ml	69	3.02 (3.6)	2.47 (2.4)	-0.544	0.1653
Leptin [ng/ml]	69	18.38 (12.7)	20.16 (14.5)	1.777	<0.05
IL-6 [pg/ml]	69	1.92 (1.2)	2.15 (1.7)	0.231	0.3208
IL-8 [pg/ml]	63	12.93 (3.6)	12.69 (2.9)	-0.239	0.6087

Endothelial Progenitor Cells

Endothelial progenitor cells (EPCs) are derived from bone marrow and function in ongoing endothelial repair. Impaired mobilization or depletion of these cells has been shown to contribute to endothelial dysfunction and cardiovascular disease progression. The number of endothelial progenitor cells in peripheral blood is being assessed to determine how numbers of these cells change in response to the program. Preliminary results are provided in Tables 10A and 10B.

Control subjects are carefully matched to Ornish participants for age, gender, and disease status, but despite this careful matching, control subjects have higher levels of EPCs at baseline. At the 12-week examination, borderline significant changes in levels of endothelial progenitor cells have been detected for Ornish participants (P=0.06) but not control subjects. By one year, neither Ornish participants nor controls exhibit a significant change in EPC levels.

Table 10A. Change in Endothelial Progenitor Cells after 12 Weeks in Ornish Program and Control Participants

Category	N	Mean Baseline (SD)	Mean Week 12 (SD)	Mean Change	P
Ornish Participants	87	0.159 (0.217)	0.114 (0.094)	-0.044	0.0614
Controls	72	0.193 (0.271)	0.171 (0.158)	-0.022	0.3745

Table 10B. Change in Endothelial Progenitor Cells after 1-Year in Ornish Program and Control Participants

Category	N	Mean Baseline (SD)	Mean One Year (SD)	Mean Change	P
Ornish Participants	50	0.173 (0.275)	0.167 (0.410)	0.0	0.9375
Controls	35	0.242 (0.376)	0.143 (0.096)	-0.1	0.1347

Gene Expression

Our work on gene expression analysis continued during the year, with continued efforts on profiling gene expression in Ornish and control participants. Gene expression in peripheral blood was examined in 231 samples (all three time points) collected from 77 participants. All samples were subjected to globin reduction using the Ambion Globin Clear kit, amplified, fragmented, hybridized to expression arrays, and scanned for gene expression analysis. In preliminary analyses, the expression intensity of each gene within each patient was converted to a rank score (the highest intensity was assigned the lowest rank). Change over time was assessed by examining how many times the rank for each gene increased or decreased. Potentially important genes were identified by determining which genes exhibited a pattern of change in Ornish participants that was significantly different from that in controls. These results suggest that fundamental molecular changes occur due to participation in the program.

The first preliminary data analysis was conducted on the gene expression data, resulting in the following abstract being presented as a poster at the Nutrition, Physical Activity, and Metabolism Conference 2008 and 48th Cardiovascular Disease Epidemiology and Prevention Annual Conference 2008, March 11-15, 2008, Colorado Springs, CO:

Changes in Global Gene Expression Profiles during a Lifestyle Change Program for Heart Disease Reversal Correlate with CAD Risk Factor Improvement

Ellsworth DL, Weyandt J, Patney HL, Love B, Burke A, Haberkorn MJ, Neatrour DM, Vernalis MN. Windber Research Institute, Windber, PA; Windber Medical Center, Windber, PA; Invitrogen, Carlsbad, CA; Walter Reed Army Medical Center, Washington, DC.

Background: Coronary artery disease (CAD) is a leading cause of death, disability, and healthcare burden. Many patients with ischemic CAD receive surgical interventions and lipid-lowering drug therapy as the main component of their treatment regime. An alternative, noninvasive approach to CAD management involves intensive risk factor modification through comprehensive lifestyle changes. Although lifestyle change is effective in improving traditional CAD risk profiles, little is known about molecular responses that may form the basis for disease progression.

Methods: Patients (n=48) participated in a prospective, nonrandomized, lifestyle change program designed to stabilize or reverse progression of CAD through dietary changes, exercise, stress management, and group support. Nonintervention controls were matched to patients based on age, gender, and disease status. CAD risk factors and risk for future coronary events were assessed over the course of one year. Global gene expression profiling was conducted on peripheral blood samples at each time point using Affymetrix U133A 2.0 arrays containing ~14,500 genes.

Results: Most patients showed significant improvements in BMI, lipids, blood pressure, and exercise capacity, as well as cardiovascular risk. In addition to physiologic responses, we identified a subset of 414 genes exhibiting highly significant changes in expression among patients in the lifestyle modification program. Of note, genes LTA4H, DPYSL2, CD36, EIF4EBP1, BRD4, and RIOK3 showed increasing expression, while levels of HLA-F decreased during the intervention. These changes in gene expression were significantly correlated with improvement in important CAD risk factors.

Discussion: These results suggest that fundamental molecular changes occur during intensive cardiovascular lifestyle modification. Differentially expressed genes may contribute to CAD risk

factor improvement because they function in diverse biological processes such as plaque stability, fatty acid transport, and blood pressure regulation. Defining genes associated with changes in CAD risk factors may improve our understanding of the molecular biology of CAD and aid in the development of more effective therapies for reducing cardiovascular risk.

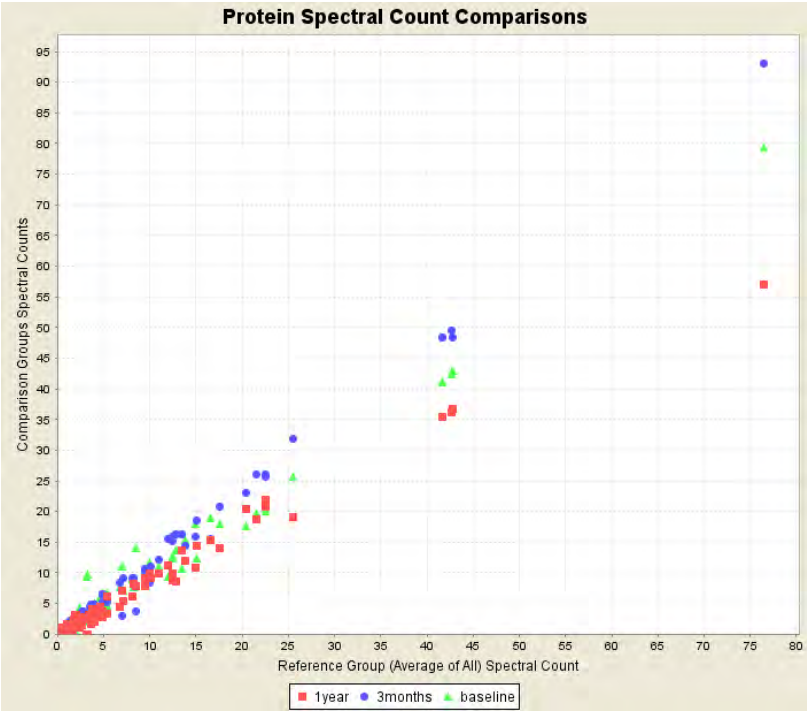
Structural and Functional Measures of Cardiovascular Health

Specific endpoints we are measuring include ejection fraction and wall motion, coronary artery calcification scores, left and right ventricular volumes, myocardial mass, stenosis sizing and vessel diameter, plaque density and differentiation of calcified versus non-calcified plaque, and tissue perfusion and viability. Data from the combination PET and coronary angiography scans are being acquired through collaboration with Dr. Josef Machac at Mt. Sinai Medical Center in New York, who is working with us in the quantification and interpretation of the huge volumes of imaging data we have acquired. To date, we have conducted on Ornish participants (includes PET rubidium, Calcium CT, and CTA scans) 57 baseline, 32 12-week, and 19 one-year scans and on control participants, 59 baseline, 19 12-week, and 14 one-year scans. As of February 2008, we will no longer offer PET/CT scanning as a testing option due to a reduction in participation and limited availability to research of the PET/CT scanner. To date we have received 156 PET rubidium, 19 Calcium CT, and 9 CTA research results. Analysis of these images are ongoing by Dr. Machac's group and the research data will continue to be analyzed in the coming year, this will help to provide a more comprehensive picture of the effects of the program on cardiac health.

Proteomics

In the current period, we have continued the proteomic analysis on 60 additional plasma samples that comprise three time points from 20 subjects (controls and Ornish participants). The samples were processed on an antibody column to remove the highly abundant proteins and the flow-through fractions were trypsin-digested and analyzed on a high performance LC/MS/MS instrument in triplicate. All datasets were processed using Bioworks software and searched against the Uniprot protein database for protein identifications. In addition to the DeCyderMS for protein expression analysis, we have used the ProteoIQ tool (beta version) to derive protein expression information from the lc/ms/ms datasets. Unlike DeCyderMS, the ProteoIQ uses the MS/MS spectral counts to calculate the protein expression from lc/ms/ms datasets. The robust integration of tools in ProteoIQ for grouping the Sequest results into biological groups (3 time points), data normalization, protein and peptide probabilities calculation, and automatically comparing and quantifying protein expression has enabled us to generate the protein expression data for individual subjects and as well as groups of subjects for three time point comparisons. The first pass of analysis using ProteoIQ was performed on 9 subjects individually, and as a group. The spectral count comparisons of proteins from this analysis are shown in Figure 2. We are continuing our efforts to acquire more proteomics data from additional plasma samples and to analyze the lc/ms/ms datasets using various tools to discern the protein expression trends between the time points. We plan to extract the relevant fields from the processed proteomics datasets for linking the peptide and protein data to the clinical database.

Figure 2 (below). Protein spectral count comparisons for baseline, three-month, and one-year examinations within a single subject (#430).



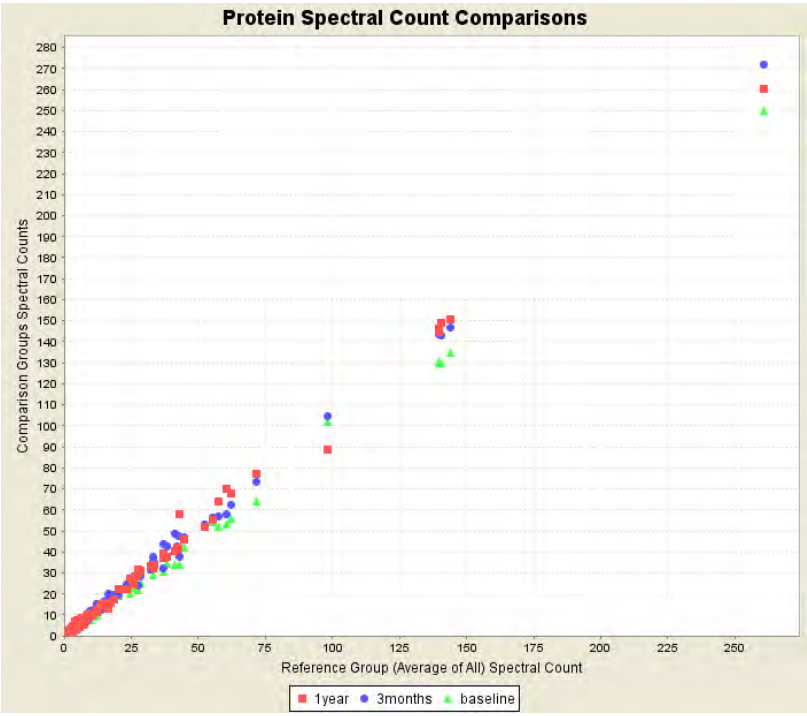


Figure 3 (above). Protein spectral count comparisons between the three time points in nine individuals (three biological replicates for each time point).

Adverse Events

All adverse events are submitted and adjudicated by the Windber Medical Center Institutional Review Board and Ft. Detrick Office of Research after review by both the Principal Investigator and Medical Monitor. There have been 50 adverse events over the course of the study, of which 28 were serious events and 22 were considered non-serious. A serious event is defined as occurring at any dose or intervention level that results in any of the following outcomes: (1) results in death, (2) a threat to life, (3) inpatient hospitalization or prolongation of existing hospitalization, (4) persistent or significant disability or incapacity, (5) causes cancer, (6) is an overdose, or (7) any medical event that requires treatment to prevent one of the medical outcomes listed above. Therefore, 31 events were considered serious due to inpatient hospitalizations and 31 were cardiac related. No deaths occurred and 7 of these adverse events were deemed to be study related and were defined as possible risks in the study consent.

2. Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal, Sub-Study for Previous Subjects in the Dr. Dean Ornish Program for Reversing Heart Disease.

The primary objective of this study is to examine associations between DNA variation (in the form of 500,000+ single nucleotide polymorphisms) and participant response to the program. We are examining the influence of innate genetic variation on overall response, quantified as the risk of future cardiac events (Framingham risk), as well as response of specific cardiovascular disease risk factors. The main hypothesis is that innate variation in genes associated with lipid metabolism, protein biosynthesis, protein modification, transcription regulation and/or cell surface receptors (or other genes) will correlate positively with response to intensive lifestyle changes involving diet, exercise, meditation, yoga and group support, which may lead to improved CHD risk factor profiles and genetic markers of coronary artery disease reversal or stabilization. Participants in this study are being recruited from previous cohorts of the Dr. Dean Ornish Program for Reversing Heart Disease at Windber Medical Center (prior to implementation of the primary Molecular Profiling Protocol described above).

Status: During the period, 182 arrays, representing 91 participants, were run on Affymetrix 500K SNP chips. Both current graduates and participants in the Sub-study (past graduates) were assayed. To date, 500K SNP data has been collected on 179 Ornish participants who have graduated from the Program. During the coming year, we will continue to run SNP assays on individuals as they graduate from the program.

As part of the developmental work with large-scale SNP genotyping, 30 500K SNP arrays (15 *Nsp* I, 15 *Sty* I) were run to examine the effect of whole-genome amplification on the ability of 500K arrays to detect copy number changes in the human genome. Ten different patients were examined, representing four different types of samples: (1) 3 patients with DNA isolated from serum (unamplified), (2) 2 patients with DNA isolated from plasma (unamplified), (3) 5 patients with DNA isolated from OCT sections (unamplified), and (4) the same 5 patients with DNA isolated from formalin-fixed, paraffin-embedded tissues (amplified). The manuscript (see reference below) summarizing our research and development activities examining whole-genome-amplification on small amounts of DNA in plasma/serum, and subsequent analysis on 500K SNP arrays was published in May 2008.

Croft DT Jr, Jordan RM, Patney HL, Shriver CD, Vernalis MN, Orchard TJ, Ellsworth DL. Performance of whole-genome amplified DNA isolated from serum and plasma on high density single nucleotide polymorphism arrays. J Mol Diagn 2008;10:249-257.

Task #7: Acute Endothelial Dependent Responses to Distinct Macronutrient Challenges Study: A Comparison of Brachial Reactivity Responses to a Low Carbohydrate/High Fat, High Carbohydrate/Low Fat, or an AHA Meal in Subjects at Risk for Coronary Heart Disease protocol.

The primary objective of this study is to evaluate the effect of three varied meals (acute macronutrient challenges) on endothelium-dependent flow-mediated dilation of the brachial artery in subjects with one or two risk factors for coronary artery disease (overweight, hypertension, hyperlipidemia, or a family history of premature coronary artery disease). Secondary objectives include assessing the affect of the macronutrient challenge on various blood biomarkers, including markers of inflammation, coagulation factors, endothelial progenitor cell numbers, and markers of NO function as well as standard lipid, glucose, and insulin levels. Endothelial dysfunction has been implicated as an early event in atherosclerosis and in the pathogenesis of coronary artery, peripheral vascular, and cerebrovascular disease. Impairment of endothelial function has been demonstrated after high glucose and high saturated fat meal challenges in healthy adults. Specifically, we are studying the acute effects of a high carbohydrate/low fat (Ornish), low carbohydrate/high fat (Atkins), and standard (American Heart Association) meals on endothelial function.

Study recruitment and intervention at the University of Maryland is complete. All 24 participants have completed all three meals for the study; all BART reads have been completed and blood samples have been collected. Dr. Vogel at the University of Maryland has read the brachial artery reactivity studies. Data for the BART study meals characteristics, baseline characteristics of BART study participants, brachial artery reactivity, and changes in the NMR lipid panel by meal were presented in last year's report. Assays for the following markers have now been completed: ADMA, hsC-reactive protein, activated factor VII-A, oxidized LDL, PYY, CCK, VCAM-1, adinopectin, ghrelin, leptin, interleukin-6 and-8, tumor necrosis factor, triglycerides, glucose, and insulin. This data is currently being analyzed at the University of Maryland.

Collaborators for this project are:

Alan Shuldiner, MD – Joslin Center for Diabetes
Robert A. Vogel, MD & Richard B. Horenstein, MD – University of Maryland School of Medicine
Wendy Post, MD – Johns Hopkins Hospital
Christie M. Ballantyne, MD – DeBakey Heart Center, Baylor College of Medicine
Dean Ornish, MD, Preventive Medicine Research Institute

Task #8: Initiate "A Feasibility Study of the Effect of Exercise Intensity on Visceral Fat" Protocol.

Status: This task has been closed to allow resourcing for more operationally related research projects.

Task #9: Initiate Influence of Exercise and Stress Management on the Metabolic Syndrome protocol.

Status: This study was terminated in the second quarter of 2007.

Task #10 Creation of a Comprehensive Cardiovascular Risk Assessment and Prevention Program (CPP).

This program was established to address the unique needs of military beneficiaries at risk for CV disease. It includes conventional and novel CV risk profiling (health assessments, labs, markers, wearable monitors) and tailored, personalized behavioral recommendations for primary or secondary prevention by an integrative team of providers comprised of a cardiologist, sleep specialist, nurse practitioners, nutritionists, stress management instructors and exercise physiologists. Validated tools to screen for and measure CV risk are part of this inclusive package. Report cards for the patient and provider as well as email notifications are utilized. The program is an adjunct to the best medical practices provided by their primary care provider. Up to 1000 patients may be enrolled each year. Some of the patients (such as nurses or medical holdovers etc) may be in subgroup programs because of unique needs. The CPP serves as a platform for ongoing translational research activities, a "virtual laboratory" for the development of best preventive practices and for CV educational and marketing materials.

Status: During 2008, customer satisfaction surveys indicated a score of 3.9 out of 4.0, continuing an excellent track record of participant satisfaction. The total number of appointments in the CPP alone during 2008 has been 2132, an average of 178 per month. This number encompassed the enrollment of 152 new patients in the CPP, doubling the enrollment from the previous fiscal year.

Of the first 92 consecutive graduates: 18 (20%) had diabetes and 19 (21%) had pre-diabetes. Significant changes (means) included: in diabetes HbA1C 7.0±1.3% to 6.1±0.47, p=0.02; in pre-diabetes glucose 104±4% to 95±10, p=0.002; in 74 non-diabetics risk of developing diabetes in 10 years improved substantially. Our integrative approach appears to be highly successful in improving glucose control in diabetes, reversing pre-diabetes (in 80%) and preventing disease onset. These published findings suggest a need to further explore our approach compared to traditional diabetes care. Randomized, controlled protocols to assess the efficacy of the CPP approach as well as CPP sub-populations compared to usual care are being developed.

The CPP has reached out to include soldiers in the Warrior Transition Brigade (WTB). In order to accommodate this subpopulation two new tracks have been developed within the CPP. The first track is tailored for soldiers and families with prolonged stays while the second track accommodates those soldiers in WTB for short durations emphasizing healthy lifestyle, sleep and stress management in a flexible half-day educational workshop.

Initial plans have been made to support patients pre and post bariatric surgery. Additionally, early collaborative efforts have been established with the Human Optimization Center by Dr. Francis O'Connor at USUHS.

Sub Task #10.1 Initiate "Validation of the ICHP Cardiovascular Risk Score" protocol.

Data previously collected on patients enrolled in the Prospective Army Coronary Calcium (PACC) and PACC Rescan projects will be reviewed. Specific information will be gathered and analyzed to give each patient a CV disease risk score according to a formula developed by the ICHP. This ICHP formula uses the Framingham model of risk prediction and adds historical factors and biochemical markers to produce a novel score predictive of CV disease risk. The goal of the study is to validate the utility of this novel ICHP scoring system by comparing the predicted risk with outcomes in this well characterized population. The primary objective of the project is to validate the predictive utility and accuracy of the ICHP CV risk score (or ICHP

score). Specifically, the goals are: a) to determine if the ICHP score correlates with cross-sectional prevalence of coronary calcium as measured in the PACC project and b) with the development of CHD events such as angina, myocardial infarction, or need for CV intervention such as coronary stenting, angioplasty, or bypass surgery. A third goal is: c) to determine the correlation of the ICHP score with coronary calcium progression as measured in the PACC rescan project. Correlation with CIMT will be performed in a PACC subset of 200 patients.

Status: Exempt protocol approved by WRAMC DCI. Change of PI request from Dr. Taylor to Dr. Modlin has been submitted. From the PACC rescan cohort, patients with a CIMT measurement were included in data analysis. For each patient, an "atherosclerosis score" was assigned according to quartiles of CAC score, CAC progression, and IMT with each ranked from 1-4, lowest to highest. This gave individual patients a possible score from 3-12. With this composite measure the top quartile of atherosclerosis score was selected. Using linear and logistic regression, the Framingham Risk Score (FRS) showed no statistically significant correlation. With the ICHP score, linear regression showed a co-efficient of 0.003 (p=0.004). Translated, this means that an increase in ICHP score by 1 point is associated with an increase in IMT of 0.3%. Using the ICHP score, logistic regression showed an odds ratio of 1.04 (p=0.01). Translated, an increase in ICHP score by 1 point is associated with increased odds of 4% for being in the top quartile for atherosclerosis score. These data are being prepared for publication.

Sub Task #10.2 Initiate Caregiver Support Program

This proposal outlines a plan to provide a comprehensive CV risk and stress reduction program for the WRAMC nursing staff, including prescriptions for therapeutic lifestyle change and stress reduction.

Project participants will complete questionnaires, state of the art lab tests and cardiovascular risk markers and will wear non regulated noninvasive wearable sensor monitoring devices to better evaluate individual CV risk and identify emotional/behavioral triggers of stress. A dedicated workshop held at the ICHP center will deliver comprehensive instruction on diet, exercise, sleep, and stress management. Follow-up over 12 weeks will include facilitated group support sessions, coaching on coping skills, tension tamer techniques, and scheduled group exercise sessions. Participants will be further engaged by telephone and email to track progress and deliver pertinent instruction and encouragement. At the end of the 12-week program, measures will be repeated to determine progress in stress reduction and changes in the CV health profile. Subsequently, participants will continue to be engaged by telehealth and coaching and those who report setbacks will be offered re-enrollment in the program. Data gathered on participants will undergo dynamic statistical modeling to yield predictive information on best lifestyle change strategies to employ for future participants. This dynamic statistical modeling will provide a more precise intervention strategy for incoming participants and allow for improved outcomes, greater efficiencies, and cost savings.

Status: We prepared and submitted a proposal for an FY08 Advanced Technology/Therapeutic Development Award. The proposal title is: "Caregiver Support Program: The Use of Advanced Technology and Lifestyle Interventions to Reduce Physical and Psychological Stress in TriService Providers."

The ICHP proposal will be a prospective randomized clinical trial of military caregivers over a 5 year period. It will expand a successfully implemented pilot project that has previously produced impressive outcomes. The pilot project was endorsed by the WRAMC Command and

Department of Nursing and included 32 nurses with high stress. The nurses completed a 12-week on-site comprehensive cardiovascular health promotion program as a non-stigmatized portal of entry for stress reduction. Baseline information on lifestyle habits, cardio-metabolic markers, and anthropometric measures were collected. Advanced technology incorporating novel noninvasive sensewear (BodyMedia) for objective physiologic measurements of energy expenditure, activity, and sleep were utilized to track progress. Demonstrated improvements were seen in job satisfaction (37% of graduates), compassion fatigue (43%), and burnout (59%). Improvements in these key indicators resulted from reduction in perceived stress (39%), enhanced sleep quality (30%), diminished sleepiness (34%) and decreased fatigue (47%). The greatest numerical improvement was in physical activity, occurring in 22 out of 32 nurses (69%). With the FY08 award, we will increase enrollment in the on-site program and develop advanced technology to include a package of web-based tools to empower caregivers to sustain their gains. These tools include an effective motivational health coach component, a novel automated personal guidance feedback system, and a customized, noninvasive physiological monitoring tracking system.

Key Research Accomplishments

- Non-Invasive Coronary Artery Disease Reversal" (CADRe) Study Protocol
    - 1 manuscript accepted; 1 manuscript in preparation

- Non-Invasive Coronary Artery Disease Reversal (CADRe) Follow-Up Study
    - Subject enrollment totaled 76 patients

- Better Adherence to Therapeutic Lifestyle Change Efforts (BATTLE) Trial
    - Approval of study addenda; Change of PI
    - Operations Manual finalized
    - Curriculum finalized
    - Data management refined
    - Recruitment and enrollment initiated; 500+ interested patients contacted or possible enrollment; 160 patients enrolled to date in screening phase; 38 patients randomized

- Dr. Dean Ornish Program for Reversing Heart Disease protocol
    - Publication in the Journal of Cardiopulmonary Rehabilitation and Prevention
    - Subject enrollment to date is 400 participants - 25 cohorts / 4 retreats
    - Age/Gender/Disease state matched control group established to compare risk factor changes

- Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal
    - Subject enrollment in the research protocols totaled 374
    - Publication in the Journal of Molecular Diagnostics
    - Poster presentation at the Nutrition, Physical Activity, and Metabolism Conference 2008 and 48th Cardiovascular Disease Epidemiology and Prevention Annual Conference 2008, March 11-15, 2008, Colorado Springs, CO
    - Comprehensive NMR lipid panel shows significant clinical improvement in Ornish participants – the average LDL-particle number (more strongly linked to CVD risk than LDL levels) improved from a clinically significant category (borderline high) to a non-significant category (near optimal)

- Participation in the Program may reduce levels of important biochemical risk factors for CAD, such as insulin, CRP, and leptin.
- Fundamental molecular changes were shown to occur in Program participants – patterns of global gene expression can differentiate baseline and one year examinations and distinguish Ornish participants from controls Initial proteomic studies on Program participants indicate that the expression of a number of plasma proteins is altered during the Program

- Cardiovascular Prevention Program (CPP):
  - Minimal risk protocol submitted to WRAMC DCI for retrospective review of CPP data collected to date.
  - 5 peer-reviewed publications from the CPP have been generated.
  - Algorithms for continuity portion of CPP have been implemented.

- Caregiver Support Program: Protocol prepared and ready for submission pending grant funding.

- Acute Endothelial Dependent Responses to Distinct Macronutrient Challenges Study: A Comparison of Brachial Reactivity Responses to a Low Carbohydrate/High Fat, High Carbohydrate/Low Fat, or an AHA Meal in Subjects at Risk for Coronary Heart Disease
  - Protocol enrollment and intervention complete
  - Brachial artery reactivity studies complete
  - NMR lipid panel complete
  - Assays for the following markers are complete: ADMA, hsC-reactive protein, activated factor VII-A, oxidized LDL, PYY, CCK, VCAM-1, adinopectin, ghrelin, leptin, interleukin-6 and-8, tumor necrosis factor, triglycerides, glucose, and insulin

Reportable Outcomes

Published Manuscripts/Abstracts:

Marshall, DA, Walizer, EM & Vernalis, MN. Achievement of heart health characteristics through participations in an intensive lifestyle change program. (Accepted for publication in the Journal of Cardiopulmonary Rehabilitation and Prevention on 3 Oct 08).

Croft DT Jr, Jordan RM, Patney HL, Shriver CD, Vernalis MN, Orchard TJ, Ellsworth DL. Performance of whole-genome amplified DNA isolated from serum and plasma on high density single nucleotide polymorphism arrays. J Mol Diagn 2008;10:249-257.

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Tschiltz N, Eliasson A, Kashani M, Vernalis M. Do dietary guidelines influence population eating habits? J Nutr Educ Behav 2008, 40:S54-S55.

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Marshall, D., Walizer, E., Vernalis, M. (2006). Enhancing achievement of healthy lifestyle habits in military healthcare beneficiaries through a lifestyle intervention program. Circulation, 113(8), e301, p. E329.

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Marshall, D., Walizer. E., & Vernalis, M. (2004). Optimal healing environments for chronic cardiovascular disease. Journal of Alternative and Complementary Medicine, 10(Supplement 1), S147-S155.

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Marshall, D., Vernalis, M., Walizer, E., Arthur, C., Northrup, G., Kashani, M., Darst, T., Turner, E., Walker, M., Greene, R., Gadhia-Smith, A., & Sundstrom, S. (2003). Early efficacy of intensive lifestyle change in patients with or at risk for coronary artery disease. Circulation, 107, e7001-e7039, p. 33.

Marshall, D**.,** Walizer, E., & Vernalis, M. (2003). Gender differences in C-reactive protein with intensive lifestyle interventions in patients with or at-risk for coronary artery disease. Circulation, 107(May 20), e131-e184, p. 19.

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Presentations (Oral & Poster):

Tschiltz N, Kashani M, Eliasson A, Vernalis M. Individuals with diabetes benefit from an integrative program using the Mediterranean diet. Food and Nutrition Conference and Exposition, Chicago, IL, Oct 2008, Poster Presentation

Kashani M, Eliasson A, Mayhew, M, Tschiltz N, Turner E, Hoffman J, Vernalis M. Optimal diabetes management using an integrative prevention model. Force Health Protection Conference, Albuquerque, NM, Aug 2008; Poster Presentation

Tschiltz N, Eliasson A, Kashani M, Vernalis M. Do dietary guidelines influence population eating habits? Society for Nutrition Education, Atlanta, GA, Jul 2008, Poster Presentation

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Conclusions

Unhealthy lifestyle behaviors are linked to the development of CHD, as well as other chronic diseases. Projections based on combined CVD risk factor impact suggest that favorable lifestyle habits could nearly eliminate the development of CHD and substantially decrease CHD morbidity and mortality. We have demonstrated that comprehensive lifestyle interventions are remarkably efficacious in reducing CVD risk factors and, in many cases, are comparable to pharmacological interventions. Future research endeavors from this project will provide new information regarding strategies to improve adoption of healthy lifestyle behaviors, the impact of lifestyle interventions on CVD risk, and the biologic mechanisms through which lifestyle changes exert their influence. Through this research, the DOD has a unique opportunity to identify and address adverse lifestyle behaviors and CVD risk factors early and make cardiovascular health a part of the military culture. A commitment to CV health could prevent cardiac events, reduce the need for costly procedures and hospitalization, improve quality of life and protect the investment of highly trained military personnel.

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2. Report Date: 12/12/2008

3. Reporting Period: Annual Report

4. Principal Investigator: COL (ret) Marina Vernalis, MC, USA

5. Telephone No.: 202-782-1555/6525

6. Award Organization: Henry M. Jackson Foundation for Advancement of Military Medicine

7. Project Title: Molecular and Clinical Based Cardiovascular Care Program

8. Current staff, role and percent effort of each on project:

STAFF MEMBER	**ROLE**	**% EFFORT**
Marina Vernalis	PI	100
Arn Eliasson	Senior Research Consultant	50
Audra Nixon	Director, Administration	100
Mariam Kashani	Director, Clinical Programs	100
Elaine Walizer	Clinical Research Coordinator	100
Linda Chrosniak	Clinical Consultant	0
Maren Mayhew	Nurse Practitioner	100
Jill Phillips	Nurse Practitioner	0
Nancy Tschiltz	Clinical Dietitian	100
Joy Halsey	Clinical Dietitian	70
Ellen Turner	Exercise Physiologist	100
Nancy Saum	Clinical Research Associate	100
Jacqueline Hoffman	Stress Management Instructor	100
TBD	Data Manager	0
Graeme Buenaflor	Health Fitness Instructor	60
Lydia Hill	Clinical Manager	100
Karla Bailey	Sonographer	100
Josephine Henderson	Clinical Admin Asst	100
Christa Caporiccio	Clinical Admin Asst	90
Jill Levin	Research Assistant	0

9. Contract expenditures to date (as applicable):

COST ELEMENTS	THIS QUARTER	CUMULATIVE
Personnel	**784,150.87**	**4,668,850.80**
Supplies	**54,167.63**	**503,738.61**
Equipment	**0**	**43,637.00**
Subcontracts	**690,529.80**	**5,229,571.81**
Indirect Costs	**127,424.01**	**793,517.63**
Total	**1,656,272.31**	**11,239,315.85**

10. Comments on administrative and logistical matters.